(<0.15 μM). To examine the kinetics of NMIIB at single-molecule level we used a dual-beam optical tweezers. A surface-immobilized bead was coated with single- (NMIIB-SH-HMM) and double-headed (NMIIB-HMM) heavy meromyosin-like molecules. We measured the lifetimes of unitary actomyosin interactions and determined the actin-detachment kinetics at two ATP concentrations. Results showed that at physiological ATP concentration (1 mM), the rate of detachment of acto-NMIIB-SH-HMM interactions was ~0.51 s-1, similar to the ADP release rate and steady-state ATPase rate reported from solution kinetic studies. Decreasing the ATP concentration to 1 μM did not alter this rate of detachment (~0.43 s-1). In case of NMIIB-HMM the detachment rates were ~0.43 s-1 (1 mM ATP) and ~0.28 s-1 (1 μM ATP). Also, we found that the power-stroke of NMIIB-SH-HMM and NMIIB-HMM were about 8 nm. No signs of processive stepping were observed in case of NMIIB-HMM. The detachment rates calculated from landing assays using NMIIB-SH-HMM-GFP and NMIIB-HMM-GFP were 0.58 and 0.57 s-1 at 1 mM ATP. The double-headed molecules were not motile, but we observed robust motility of minifilaments of full-length NMIIB-GFP. We will discuss our single-molecule results from the perspective of the essential cellular functions of NMIIB in cell locomotion, tension generation and maintenance.

691-Pos Board B491

Non-Linear Elasticity of Skeletal Myosins is Essential for Collective Force Generation in Muscle

Motoshi Kaya, Hideo Higuchi.

Muscle contraction occurs through the rotation of the myosin heads pulling actin filaments past myosin filaments. In order to achieve contractions efficiently, it is crucial for myosins to generate force collectively and to minimize the interference between motors. In the previous studies, it was suggested that the elasticity of myosin is linear so that myosins generate the large drag force opposing to muscle contraction if negatively strained. However, none of the previous studies has investigated the elasticity of single myosin explicitly in the negative strain region. Therefore, we investigated the elasticity of single skeletal myosins in both the positive and negative strain directions. In the absence of ATP, single myosins embedded in synthetic myosin-rod filaments were tightly bound to actin filaments and were stretched and shortened repeatedly by oscillating the two trapped beads on the both ends of acting filament manipulated by optical tweezers. The elasticity of myosins was characterized by obtaining the force-displacement curves, where forces on myosin heads were estimated by measuring the displacement of two beads, and displacements of myosin heads were obtained by tracking the position of quantum dots attached to the actin filament. We found that the elasticity is non-linear, in which stiffness is high (~2.8 pN/nm) and low (~0.02 pN/nm) in the positive and negative strain regions. The non-linear elasticity ensures high force generation with a small stretching of the elastic portion, and minimizing the drag force of negatively-strained myosins. Furthermore, the estimates of the actin sliding distance by the non-linear elasticity model were more consistent with experimental results observed in 20μM ATP than those by the linear elasticity model. Therefore, the non-linear elasticity might be an essential mechanical property of single myosin designed for the collective force generation in muscle.

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Observation of Mechanical Process for Myosin-Ii by a Dark-Field Microscopy Combined with an Optical Trap

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Myosin-II molecules are self-assembled and form a muscle. Individual myosin heads in muscle does not seem to interrupt each motion and fully exhibit their function. Myosin heads are connected to the backbone filament via S2-coiled-coil (~ 60 nm) with non-linear elasticity. Motor domain undergoes Brownian motion (probably > 10 nm fluctuation), but small working stroke (~ 10 nm) by the single myosin head is fully converted to macroscopic sliding motion of muscle. Thus, flexibility enabling myosin head's Brownian motion reduces molecular friction in muscle, but how small working stroke within the thermal noise can be fully converted to macroscopic motion is still unclear.

To answer the question, we should visualize the Brownian process and transition from pre-working state to post-working state. Detection for the process has succeeded using micro-needle method (Kitamura et al., 1999), but detail analysis at modulated conditions was hard due to the difficulty for the method.

Here we used dark-field microscopy combined with an optical trap and observed interaction between myosin II S-1 and actin. S1 was tagged with small optically-trapped particle (60 nm gold or 200 nm polystyrene bead), which enabled us to quantify non-processive steps and processive steps concerning a few myosin heads with rapid response time. We will discuss these results and trials of mechanical modulation to the myosin head at sub-milli second time scale to examine strain-dependent force generation like myosin-VI (Iwaki et al., 2009).

693-Pos Board B493

Making Sense of the Motility Assay: Automated Video Analysis and Molecular Model Based Inference

Lennart Hilbert, Genevieve Bates, Horia N. Roman, Michael C. Mackey, Anne-Marie Lauzon.

Background: We present computational analysis and inference tools to assess actomyosin interaction in the motility assay.

Methods/Results:

Analysis: Individual actin filaments are automatically detected and tracked by area and position similarity in consecutive video frames. Filament velocities (V) are calculated as distance traveled over elapsed time (T). For individual filaments, the software measures filament length (L), velocity (V), number of immotile frames. For the overall video, the breakage of filaments is monitored. Velocity averages were found to be linearly related to values obtained manually using Scion Image v.4.02 (NIH Image) (proportionality factor:0.96 ± 0.10, from 8 videos).

Model: A model was developed that incorporates actomyosin binding sites with independent kinetics (2 states:attached/detached, Poisson escape statistics: mean attached time τ_{on} and mean detached time τ_{off}, binding site distance (B)). The mean filament velocity is $V(L) = V_{max}[1-P_{off} \perp (L/B)]$, with $P_{off} = \tau_{off} /(\tau_{on}+\tau_{off})$, the probability for a single binding site to be idle. Numerical analysis of the variance suggests that the standard deviation (σ) over mean V is $\sigma/V = C/\mathrm{sqrt}(T*L)$, C=constant.

Fitting: Fitting mean velocity V(L) and C to data allows the inference of V_{max}, P_{off}, τ_{on}. We simulate filament data for known parameters, reverse fit these parameters, and for 6 simulated data sets (each holding 40 filaments) we find the estimated parameter values to deviate from the input by 6%, 20%, and 20%, respectively.

Benefits/Implications: Our automated analysis software provides: 1.High filament counts 2.Detailed and reliable tracking of single filament motion 3.Flexibility to meet the specific needs of the experimental operator. Small amounts of motility data suffice to fit three important kinetic parameters. The model predicts increased variance for slow myosin types, as is observed for smooth muscle myosin.

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7 Amino Acids can Make the Difference: Chemical Cycle Kinetics Connect Smooth Muscle Myosin's Loop1 Alterations to its Dynamical Differences

Lennart Hilbert, Michael C. Mackey, Anne-Marie Lauzon.

Background: The (+) and (-)insert myosin isoforms present in smooth muscle differ by a seven amino acid sequence. The sequence, which is present only in the (+)insert isoform, is part of surface loop1, which is involved in ADP/ATP binding to and release from myosin's catalytic pocket.

We use a chemical cycle kinetics model of the smooth muscle actomyosin interaction to explore the effects of the loop1 difference on actomyosin kinetics. We connect differences in the nucleotide binding/release energy barriers with differences in cycling rate and on-time. The model also provides a framework for testing other molecular hypotheses about actin-myosin interaction kinetics of single binding sites.

Methods: 1.We constructed a kinetic cycle model, that accommodates the essential aspects of the interactions occurring at one actin-myosin binding site. The effective transition rates between the actomyosin cycle states incorporate the free energy differences, energy barriers, substrate concentrations and load on the filament. The diagram method gives an analytic expression for the actomyosin on-time (τ_{on}) and probabilities of occupation of a tight-bound state.

2.We adjusted the model parameters, so the model results match motility assay filament velocities (V) for (-)insert isoform from literature($V=d/\tau_{on}$, where d is the myosin step size).[Baker et al. J Biol Chem **278**(31):28533-28539]

3.We decreased the energy barrier for nucleotide binding/release till a two-fold increase in V was achieved. This two-fold increase of V is one generally accepted feature of the (+)insert isoform.

Results/Discussion: A $0.9k_BT$ loop1 energy barrier decrease can account for a two-fold increase in V. It also results in 87% actin binding sites tightly bound for (-)insert, compared to 76% for (+)insert. This might contribute to latching behavior in smooth muscle with high (-)insert content.NIH R01-HL 103405-01.

695-Pos Board B495

Phosphate Enhances Actin Filament Velocity at Low pH in an *in vitro* Motility Assay

Edward P. Debold, Matthew Turner, Jordan C. Stout, Samuel C. Walcott.

Elevated levels of phosphate (P_i) and decreased pH are believed to contribute to muscular fatigue by inhibiting the function of myosin; however the molecular basis of this inhibition remains poorly understood. The *in vitro* motility assay was used to systematically examine the effects of elevated P_i (0 vs. 30mM)